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Agronomic Evaluation of the Results of Selection within Late-Maturing *Dactylis glomerata* Populations

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Abstract: Selection from novel orchardgrass (*Dactylis glomerata* L.) germplasm sources resulted in the development of a late-maturing orchardgrass population. This population comprises 58 families that were evaluated with 5 commercial cultivars under frequent and infrequent harvest intervals at two Cache County, UT, USA field locations during 2013 and 2014. The objective of this study was to characterize the performance of individual families when compared to check cultivars Intensive and Latar. Across locations and harvest intervals, individual families produced greater herbage dry mass and nutritive value than the check cultivars did, i.e., up to 12% greater herbage dry mass than that of the highest check, Intensive, and 1% (neutral-detergent-fiber digestibility) to 14% (water soluble carbohydrates) greater forage quality than that of the corresponding highest check cultivar. However, there were substantial genotype-by-environment interactions between families and locations, but not harvest intervals. Because of this, results were analyzed across harvest intervals but within locations. Within each location, there were families that possessed similar or greater maturity, herbage dry mass, in vitro true digestibility, and neutral-detergent-fiber digestibility at both locations. Overall, on the basis of the performance of its component families, this late-maturing orchardgrass population exhibited potential for developing improved cultivars.

Keywords: biomass; cocksfoot; grazing; hay; herbage; nutritive value; orchardgrass



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1. Introduction

Dactylis glomerata L., commonly known as orchardgrass or cocksfoot, is a primary perennial-forage grass in temperate regions of the world. Late-maturing orchardgrass genotypes are particularly important in mixtures with forage legumes, such as alfalfa (*Medicago sativa* L.), because the flowering times of both species are complementary [1]. Thus, under hay production management in temperate regions, late-maturing orchardgrass grown with alfalfa results in harvesting times that maximize the quality and productivity of both species [2]. Late-maturing orchardgrass also provides some management flexibility to producers in spreading out harvesting requirements across a longer period. The lower forage productivity of later-maturing orchardgrass can be beneficial in legume mixtures because of lower competition with the legume [3]. However, when planted as a monoculture, late-maturing orchardgrass germplasm always produces less herbage dry mass (HDM) for a unit area.

While critical to sustainable livestock production, perennial-grass breeding in general, and orchardgrass breeding specifically, has decreased over the last few decades [4]. In many instances, cultivars that are decades old and often lack genetic purity are still common in the commercial seed market [4]. Thus, there is an important need to develop improved orchardgrass cultivars that maximize forage productivity and nutritive value. These cultivars also need to address the different methods of forage harvest from grazing to hay or ensiling. Evaluations of genetically improved late-maturing orchardgrass germplasm found that newer late-maturing cultivars do not cumulatively show improvement over older

orchardgrass cultivars [5]. These limited genetic gains for agronomic traits also necessitate the evaluation and development of genetically novel orchardgrass populations [6].

Recent germplasm evaluation and enhancement resulted in the development of a novel late-maturing orchardgrass germplasm with significant levels of genetic variation for several agronomically important traits [7,8]. This study documents the performance of 58 families derived from this broad-based late-maturing orchardgrass population and 5 commercial cultivars. The 58 families and 5 cultivars were evaluated for HDM, maturity (MAT), and nutritive value at two northern Utah field sites under both frequent- and infrequent-harvest-interval schedules.

2. Materials and Methods

2.1. Half-Sib Families

In 2011, open-pollinated seed was harvested from 58 plants originating from 7 orchardgrass germplasm sources (Table 1) [7]: IADG103, IADG105, Latar-Select, Paiute-Select, UTDG101, and UTDG102. The harvested seed was cleaned and then used to seed sward plots in August 2012.

Table 1. Fifty-eight *Dactylis glomerata* half-sib family source populations and five commercial cultivars, including number (N) or families corresponding to each population and cultivar.

Family	Type	N	Source
IADG103 (I3)	Half-sib families	3	[7]
IADG105 (I5)	Half-sib families	4	[7]
Latar-Select (LaS)	Half-sib families	8	[7]
Paiute-Select (Pa)	Half-sib families	2	[7]
PI538922 (P5)	Half-sib families	20	[9]
UTDG101 (U1)	Half-sib families	13	[7]
UTDG102 (U2)	Half-sib families	8	[7]
cv. AC Killarney (Ki)	Cultivar	1	Agriculture and Agri-Food Canada
cv. AC Kootenay (Ko)	Cultivar	1	Agriculture and Agri-Food Canada
cv. Baraula (Ba)	Cultivar	1	Barenbrug USA
cv. Intensive (In)	Cultivar	1	Barenbrug USA
cv. Latar (La)	Cultivar	1	[10]

2.2. Experiment Locations and Establishment

Swards were established at two Cache County, UT, USA sites: (1) Lewiston (41.95° N 111.88° W; 1373 m above sea level; 472 mm annual precipitation; Kidman and Lewiston fine sandy loam soil (coarse-loamy, mixed, superactive, mesic Calcic Haploxerolls), and (2) Millville, UT (41°41'52" N, 111°49'53" W; 1378 m above sea level; 432 mm annual precipitation; Nibley silty clay loam soil (fine, mixed, mesic, Aquic Argiustolls)). There were two separate sets of plots at both sites: (1) frequent and (2) infrequent harvest intervals. The frequent-harvest plots were intended to simulate a grazing management, and the infrequent-harvest plots were intended to simulate a hay management. Thus, the study comprised four site-treatment combinations: (1) Lewiston-Frequent Harvest, (2) Lewiston-Infrequent Harvest, (3) Millville-Frequent Harvest, and (4) Millville-Infrequent Harvest. Each environment consisted of 96 plots.

Because of seed and space limitations, the study was designed as an augmented design within each harvest interval at both locations. In addition to the 58 families, five cultivars or breeding populations were included. Cultivars Intensive and Latar were the check cultivars. The three other cultivars were used for comparison and as design fillers. The augmented design followed the approach employed by Riday and Brummer [11]. The plots in each of the four environments were arranged in 8 incomplete blocks, each consisting of 12 plots for a total of 96 plots. The two check cultivars were replicated once each per incomplete block to allow for estimations of experimental error and mean separation.

Unfortunately, a planting error at Millville caused a deviation from the allocation of check cultivars in each incomplete block. However, at least one check cultivar was present in each incomplete block, and it was used to estimate experimental error. This arrangement of the two check cultivars deviated from the optimal augmented design, but the result was similar to that of accepted incomplete block designs in which differences between entries are determined with differing levels of precision [12]. With at least one check cultivar in each incomplete block, stand errors of mean differences were calculable, although with different levels of precision. The other families and cultivars were then randomly assigned to the remaining plots in each environment. Because there were more plots than entries, empty plots following randomization were seeded to a cultivar, but were not considered in the experimental analysis.

Each sward had an area of 2 m² (1 m wide × 2 m long) and consisted of five seeded rows spaced 0.25 m apart. There was also a 0.5 m space between adjacent plots in the same row. In August 2012, seeding was performed with a Hegge cone seeder (Wintersteiger Inc., Salt Lake City, UT, USA) at a rate of one pure live seed·linear cm⁻¹. Immediately following plot seeding, supplemental irrigation was applied to the plots to ensure full establishment (100% germination). After seedling emergence, supplemental irrigation was continued at a rate of ~30 mm·wk⁻¹ through September to ensure stand establishment. All plots at both locations were mowed to a 100 mm stubble height following plant dormancy in the late fall of 2012.

Each environment annually received 56 kg N fertilizer ha⁻¹ in the form of 31-0-0. There were three applications of N fertilizer per year in early spring, following the first harvest, and following the third (frequent) or second (infrequent) harvest. Supplemental irrigation was applied weekly following the first harvest in each environment at a rate to approximately replace the amount of weekly evapotranspiration.

The first frequent harvest for both years was timed when plots reached a height of approximately 250 mm. The first infrequent harvest for both years was when plots had reached the booting to early ear-emergence stages of growth. The frequent-harvest plots were harvested 7 times at approximately 23-day intervals in 2013 and 5 times at approximately 31-day intervals in 2014. The difference in harvest numbers between years was due to a midsummer irrigation failure at the Lewiston location in 2014. The infrequent-harvest plots were harvested 4 times at 42-day intervals in both 2013 and 2014. Harvesting was performed using a sickle-bar-style forage harvester (Swift Machine and Welding Ltd., Swift Current, SK, Canada). Plots were cut to a 100 mm stubble height. All biomass from each plot was weighed with the built-in scale on the harvester. Approximately 300 g samples were hand-collected, weighed, and then dried in a forced air drier at 60 °C for 3 days. Following drying, the samples were reweighed, and the ratio of dry to wet weight was used to adjust the plot HDM values. These samples were subsequently ground to pass a 1 mm screen and scanned by near-infrared reflectance spectroscopy (NIRS; Foss, Hilleroed, Denmark). NIRS scan values were then used to estimate the values of crude protein (CP), *in vitro* true digestibility (IVTD), neutral-detergent-fiber digestibility (NDFD), and water-soluble carbohydrate concentration (WSC) on the basis of a percentage of dry matter. The used equation was NIRS Consortium equation 18gh50.eqa [13]. Maturity data were only collected prior to the first harvest in each year, and not for the frequent harvests in 2013. A visual rating system was used to rate the growth stage of the plants in each plot. A rating of 1 corresponded to very late plants that were at the flag-leaf-extension to swollen-boots stages, and a rating of 5 corresponded to very early plants that were at the complete ear-emergence to early-anthesis growth stages. Other rating values represented intermediate growth stages.

Data were analyzed as multienvironmental augmented design using a linear mixed model approach with the lme4 package [14] of statistical software R [15]. Experiment- and location-wide least-squares means were estimated by considering the main effects of years, locations, harvest frequencies, orchardgrass families, and the corresponding interactions as fixed effects, and the remaining main effects and interactions as random

effects. Spatial variation was controlled using the approach of Smith and Casler [16], and the year \times incomplete block effect was used to control for the repeated measures taken from each plot across years. Although the families were half-sib because they arose from selection, and interest was in their specific performance and not as representative samples of the population, they were considered to be a fixed effect. The emmeans package was used to estimate means for each factor [17]. Phenotypic correlations among traits were estimated using the cor.test command of R. Differences were considered to be significant at the $\alpha = 0.05$ level.

3. Results and Discussion

3.1. Year, Location, and Harvest-Frequency Effects

Differences among the levels of the main effects of year, location, and harvest frequency were significant for all traits except for NDFD and year (Table 2). The 2013 production year resulted in earlier MAT, lower HDM (−12%), but greater CP (5%), IVTD (2%), NDFD (3%), and WSC concentration (2%) than those in the 2014 production year. The Lewiston location resulted in later MAT, greater HDM (68%), but lower CP (−11%), IVTD (−4%), NDFD (−2%), and WSC concentration (−25%) than those of the Millville location. The frequent harvest interval resulted in later MAT (−44%), lower HDM (−18%), and greater CP (22%), IVTD (3%), NDFD (2%), but lower WSC concentration (−11%) than those of the infrequent harvest interval.

Table 2. Mean trait values corresponding to main effects of year, location, and harvest frequency. Traits were maturity (MAT; 1, very late to 5, very early), herbage dry mass (HDM; Mg ha^{−1}), crude protein (CP; g kg^{−1}), in vitro true digestibility (IVTD; g kg^{−1}), neutral-detergent-fiber digestibility (NDFD; g kg^{−1}), and water-soluble carbohydrates (WSC; g kg^{−1}), measured on 58 orchardgrass families and five commercial cultivars during 2013 and 2014 at Lewiston and Millville, UT under frequent and infrequent harvest intervals. Values, in the same column and for the same effect, followed by different letters differ at least at the 5% α level based on least-significant difference.

Effect	MAT	HDM	CP	IVTD	NDFD	WSC
Year						
2013	2.6 ^b	17.8 ^b	183 ^a	850 ^a	742 ^a	58 ^a
2014	2.5 ^a	20.3 ^a	175 ^b	834 ^b	721 ^a	57 ^b
Location						
Lewiston	2.3 ^a	23.8 ^a	169 ^b	827 ^b	722 ^b	51 ^b
Millville	2.7 ^b	14.2 ^b	189 ^a	858 ^a	740 ^a	64 ^a
Harvest Frequency						
Frequent	1.8 ^a	17.1 ^b	197 ^a	855 ^a	739 ^a	54 ^b
Infrequent	3.2 ^b	20.9 ^a	161 ^b	829 ^b	723 ^b	61 ^a

The statistical modeling of the traits included the two- and three-way interaction effects among year, location, and harvest interval. Year \times location and year \times harvest-interval interactions were significant model components for each trait except for MAT. The year \times location \times harvest-interval interaction was a significant model component for each trait except for MAT and NDFD. While there is no way from this dataset to determine the magnitude of this effect, the differences among main year effects may be partially attributable to the differences in days between harvest in each year due to the irrigation failure. However, due to the perennial nature of orchardgrass necessitating its survival and production across years, and the unpredictability of given years, the effect across years rather than within years is most important; thus, results are presented as means across the main year and interaction effects [18]. Location \times harvest interval was a significant model component for HDM, CP, and WSC concentration. Among location \times harvest-interval interactions, Lewiston \times Infrequent Harvest (26.2 Mg ha^{−1}) resulted in greater HDM than

that of Lewiston \times Frequent Harvest (21.5 Mg ha⁻¹), which in turn resulted in greater HDM than that of the two other environments. Millville \times Frequent Harvest (210 g kg⁻¹) resulted in greater CP than that of Lewiston \times Frequent (185 g kg⁻¹), which possessed greater CP than those of the two other environments. Millville \times Infrequent (72 g kg⁻¹) resulted in the greatest WSC concentration, followed by Millville \times Frequent (56 g kg⁻¹), which was greater than that of either of the two other environments.

While statistically significant, the difference in MAT between Lewiston and Millville locations was small (only 0.4 on the 1 to 5 scale) and of little biological significance. However, the differences between the two locations for the remaining traits were larger and of greater biological significance. The nearly 70% increase in HDM at the Lewiston location was particularly notable. The two locations are approximately 30 km apart and in the same mountain valley. Under irrigation, the Lewiston location is rated as prime farmland, while the Millville location is rated as a lower farmland of statewide importance [19]. Similarly, the Lewiston location is rated as a 2w irrigated capability class or subclass with only potentially moderate limitations, while the Millville location is rated as a lower 3w on this scale with potentially severe limitations. The Lewiston location (5 to 6) also rated higher than Millville did (4.5 to 5) on yields of irrigated crop ratings. Climate during 2013 and 2014 was similar at both locations, which was expected on the basis of their proximity. Annual precipitation and mean temperature for 2013 were 290 mm and 7.7 °C for Lewiston, and 267 mm and 7.6 °C for Millville; 2014 values were 463 mm and 9.3 °C for Lewiston, and 452 mm and 8.9 °C for Millville [20]. Thus, outside of microclimatic differences inherent to each location, the primary difference between locations was soil type. Orchardgrass performed better under sandier Lewiston conditions than under higher-clay-content Millville conditions. This may have been due to the decreased ability of orchardgrass roots to mine tightly bound water from smaller pores in the higher-clay-content Millville soil [21]. Although the study only included two locations, they are reflective of irrigated farms within the Intermountain United States. Despite the improved HDM of orchardgrass at Lewiston, CP, IVTD, and NDFD values were substantially higher at Millville. This is not surprising because of the long known negative relationship between biomass and the nutritive value in perennial grasses, including orchardgrass [22]. Nevertheless, substantially higher WSC concentrations at Lewiston were contrary to this evidence, although the relationship between WSC and HDM is complicated and environment-specific [23]. Additionally, perennial-forage-grass-breeding programs successfully developed cultivars with high levels of both HDM and WSC [6].

The greater HDM of the infrequent harvest interval is the result of increased time to photosynthesize and accumulated biomass prior to defoliation. The greater CP, IVTD, and NDFD values of the frequent harvest interval were due to harvesting at an earlier stage of reproductive maturity before cell-wall components greatly increased and nutritive value declined [24]. The increased WSC concentration of the infrequent harvest interval was also presumably the result of the increased length of photosynthesis and the accompanying accumulation of higher levels of photosynthate [25].

3.2. Orchardgrass Family Effects

When evaluated across years, location, and harvest intervals, there was strong evidence for differences among families for all traits but CP (Table 3). The statistically highest grouping of families for MAT (MAT scores ≤ 1.9) were the Baraula cultivar, 12 families from the PI538922 population, 4 families from the UTDG101 population, and 1 family from the Latar-Select population (Supplemental Table S1). The statistically highest grouping of families for HDM (HDM values ≥ 19.7 Mg ha⁻¹) were the AC Killarney cultivar, two IADG103 families, four Latar-Select families, two PI538922 families, one Paiute-Select family, three UTDG101 families, and five UTDG102 families. The statistically highest grouping of families for CP (CP values ≥ 181 g kg⁻¹) were the AC Kootenay and Latar cultivars, one family from the IADG103 population, one family from the IADG105 population, two populations from the Latar-Select population, six families from the PI538922 population, eight families

from the UTDG101 population, and four families from the UTDG102 population. The statistically highest grouping of families for IVTD ($IVTD \geq 848 \text{ g kg}^{-1}$) were the AC Kootenay cultivar, 1 family from the IADG103 population, 13 families from the PI538922 population, and 2 families from the UTDG101 population. The statistically highest grouping of families for NDFD ($NDFD \geq 741 \text{ g kg}^{-1}$) were the AC Killarney, AC Kootenay, and Latar cultivars, 1 family from the Latar-Select family, and 10 families from the PI538922 population. The statistically highest grouping of families for WSC ($WSC \geq 62 \text{ g kg}^{-1}$) were one IADG103 family, one Latar-Select family, nine PI538922 families, and two UTDG101 families.

Table 3. Summary of mean experiment-wide values corresponding to 58 orchardgrass families and 5 commercial cultivars evaluated in 2013 and 2014 at Lewiston and Millville, UT under frequent and infrequent harvest frequencies. Values represent the family with the highest and lowest means for each trait, the means of cultivars Intensive and Latar, the overall trait mean, and the corresponding least significant difference.

Summary Values	Maturity (1—Very Late to 5—Very Early)	Herbage Dry Mass (g kg^{-1})	Crude Protein (g kg^{-1})	In Vitro True Digestibility (g kg^{-1})	Neutral- Detergent-Fiber Digestibility (g kg^{-1})	Water-Soluble Carbohydrates (g kg^{-1})
High family	1.2	21.3	190	854	759	66
Low family	4.1	15.6	170	831	705	47
Intensive	2.1	19.1	179	838	719	58
Latar	3.2	17.0	187	844	743	58
Mean	2.5	19.0	179	842	731	58
Least significant difference (5%)	0.8	1.9	10	7	19	5

Although there was little evidence of correlation among traits (only correlations between IVTD and NDFD ($\rho = 0.62$), and between IVTD and WSC ($\rho = 0.59$) significantly differed from zero). The statistically highest group for multiple traits comprised 27 families (Supplemental Table S1). Ten were high for 2 traits, 11 for 3 traits, 5 were high for 4 traits, and 1 was high for 5 traits (PI538922_17 for MAT, HDM, CP, IVTD, WSC). None of the families was high for all six traits.

Complicating the results were the significant location \times family and location \times harvest-interval \times family interactions. The location \times family interaction was significant for HDM, CP, IVTD, NDFD, and WSC. The location \times harvest-interval \times family interaction was only significant for MAT and IVTD; for IVTD, the Type I sums of squares pertaining to the location \times harvest-interval \times family interaction were smaller than the sums of squares corresponding to the location \times family interaction. The harvest-interval \times family interaction was only significant for IVTD. Thus, the two-way location \times family interaction was the predominant interaction with family. Correlation estimates between the two locations for the two traits also evidenced the lack of association between the locations. The correlation between MAT values at both locations differed from zero ($\rho = 0.62$), but all other correlations were smaller than 0.40 and did not significantly differ from zero. Despite this, there was evidence of highly performing families at both locations (Supplemental Table S1). Eight families were among the latest-maturing at both locations (seven PI538922 families and one UTDG101 family). Eleven families were among the families with the greatest HDM at both locations (one IADG103 family, one Latar-Select family, one PI538922 family, one Paiute-Select family, three UTDG101 families, and four UTDG102 families). AC Kootenay was the only family with high CP at both locations. AC Kootenay and six PI538922 families were among families with the greatest IVTD at both locations. AC Kootenay, three PI538922 families, and one UTDG102 family were among families with the greatest NDFD at both locations. None of the families possessed high WSC at both locations. AC Kootenay and five PI538922 families possessed high trait values for multiple traits at both locations. Thus, while each of the original germplasm sources provided important genetic variation to this late-maturing germplasm, PI538922 families were particularly important for delaying MAT and increasing nutritive value.

On the basis of the limited correlation between locations for the same trait and the limited number of families that were in the statistically highest grouping for a trait at both

locations, the crossover-genotype \times environment interaction was the primary cause of the interaction [26]. This crossover interaction makes selection for adaptation across a large target environment difficult and arrives at the heart of the long-running breeding question of whether selection should be based within or across macro-environments [27]. Plant-breeding efforts ideally focus on relatively homogeneous individual macro-environments for specific adaptation. This method of plant breeding can capture positive aspects of genotype \times environment interactions by developing different varieties that are specifically well-adapted to specific regions [28]. Due to structures in the commercial seed market, perennial-forage-grass breeding requires the broader approach. Seed sales are substantially lower than those for other agricultural crops, seed increase is logistically difficult and expensive, genetic gains are slow and limited, and companies market varieties across large geographic regions [6,29]. Because of those limitations, the most likely approach for improvement within this population is selection for breeding materials that exhibit high performance across environments and for multiple traits. In our study, none of the included cultivars exhibited consistently high performance for all traits. However, the five families that exhibited high performance for four of the traits and the PI538922_17 family that exhibited high performance for five traits are promising breeding materials for genetic gain through selection in this population.

On the basis of the resulting F test from the mixed model analysis, this study showed that, in this population, the agronomic performance of individual genotypes did not interact with harvest interval. This finding facilitates selection for improved orchardgrass across harvest management within this population, but is not uniform in all perennial-forage-grass types and populations [6]. This interaction between family and location confounds selection within this population for wide adaptation, but it is the normal state of orchardgrass genetics [4]. Overall, this late-maturing orchardgrass population exhibited great potential for population improvement under frequent and infrequent harvest intervals, and already outperforms the included late-maturing orchardgrass check cultivars for most agronomic traits.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11071362/s1>, Table S1: Mean values corresponding to 58 orchardgrass families and five commercial cultivars evaluated in 2013 and 2014 at Lewiston and Millville, UT under frequent and infrequent harvest frequencies.

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